REMARKS

Claims 1, 4, 9, 20, 23, 28, 39, 42, and 47 have been amended. New claims 58-73 have been added. Claims 1-4, 9, 12, 15, 20-23, 28, 31, 34, 39-42, 47, 50, and 58-73 are pending in the present application.

It is respectfully submitted that the present amendment presents no new issues or new matter and places this case in condition for allowance.

I. The Rejection of Claims 1-4, 9, 12, 15, 20-23, 28, 31, 34, 39-42, 47, and 50 under 35 U.S.C. § 112, Second Paragraph

Claims 1-4, 9, 12, 15, 20-23, 28, 31, 34, 39-42, 47, and 50 stand rejected under 35 U.S.C. § 112, second paragraph, as being indefinite on several grounds.

Ground 1: The Office Action states that the term "heterologous biological substance" in claim 1 is vague and indefinite because it is unclear what substance other than a protein could be made by the claimed method. The Examiner has suggested amending the claim to recite "heterologous protein" in lieu of "heterologous biological substance". This rejection is respectfully traversed.

The phrase "first nucleic acid sequence directing synthesis of a heterologous biological substance" encompasses one or more genes involved in the production of the biological substance. The heterologous biological substance may be encoded by a single gene, *e.g.*, an enzyme; the product of the gene may be involved in the synthesis of the biological substance such as a biopolymer, *e.g.*, hyaluronic acid; or a series of genes composing a biosynthetic or metabolic pathway may be involved in the synthesis of biological substance such as a metabolite.

To make it clear what substance other than a protein could be made by the claimed method, claim 1 has been amended to recite: "the mutant cell comprises a first nucleic acid sequence encoding a heterologous protein, wherein the heterologous protein is the heterologous biological substance or the heterologous protein is involved in the synthesis of the biological substance".

Ground 2: The Office Action states that the phrase "comprising a modification of at least one of the genes *cypX* and *yvmC*" in claim 1 is vague and confusing because the claim should provide structural properties, *e.g.*, *cypX* comprising the nucleotide sequence set forth in

SEQ ID NO:1 and *yvmC* comprising the nucleotide sequence of SEQ ID NO:8, to adequately define the genes.

Applicants respectfully point out that the nucleotide sequence of the *Bacillus subtilis yvmC* gene is found in SEQ ID NO: 3. SEQ ID NO: 8 is the deduced amino acid sequence of the *yvmA* gene. Applicants have amended claim 1 to recite: "wherein the *cypX* gene comprises the nucleic acid sequence of SEQ ID NO: 1 or comprises a nucleic acid sequence having at least 70% homology to SEQ ID NO: 1, and the *yvmC* gene comprises the nucleic acid sequence of SEQ ID NO: 3 or comprises a nucleic acid sequence having at least 70% homology to SEQ ID NO: 3". Support for this amendment is found on page 7, line 17, to page 8, line 5, of the specification.

Ground 3: The Office Action states that the phrase "comprising a modification of at least one of the genes *cypX* or *yvmC*" in claim 1 is vague and indefinite because it is unclear that a mutation of just one of the genes would result in cessation of the production of red pigment, that both these genes are found in the strains, and that any *Bacillus* strain, other than *B. subtilis,* comprises these genes. This rejection is respectfully traversed.

While both genes of a *cypX-yvmC* operon are required to produce a red pigment, either the *cypX* gene or *yvmC* gene, or both genes, can be mutated to prevent production of the red pigment. Example 2 demonstrates the deletion of both of the *Bacillus subtilis cypX* and *yvmC* genes with loss of red pigment production, Example 3 demonstrates the deletion of the *Bacillus subtilis cypX* gene with loss of red pigment production, and Example 4 demonstrates the deletion of the *Bacillus subtilis yvmC* gene with loss of red pigment production. Consequently, the phrase "comprising a modification of at least one of the genes *cypX* or *yvmC*" means that either the *cypX* gene or *yvmC* gene, or both genes, can be inactivated by mutation, *e.g.*, disruption, deletion, etc. While the *cypX* gene and *yvmC* gene were isolated from *Bacillus subtilis* (see Examples 1 and 2), primers based on the *cypX* gene from *Bacillus subtilis* were used to clone by PCR the *cypX* gene from *Bacillus licheniformis* and delete a portion of the gene to prevent formation of the red pigment (see Example 6).

Ground 4: The Office Action states that the term "modification" in claim 1 should be changed to "mutation" and specifically recite that the mutation results in loss of red pigment production because claim 1 does not directly correlate the mutation to the loss of pigment.

Claim 1 has been amended according to the Examiner's suggestion.

Ground 5: The Office Action states that claim 1 is also vague and indefinite because it

reads on using a cell with unmodified *cypX* or *yvmC* genes transformed with a vector comprising mutant *cypX*/*yvmC* genes and reads as if the desired substance to be produced is actually linked to the mutated *cypX* or *yvmC* gene, when the specification teaches it is a cell comprising a mutated red pigment gene into which a recombinant vector is transformed and the claim must be amended to appropriately convey this concept. This rejection is respectfully traversed.

The Office Action asserts that the specification teaches that the cell comprises a mutated red pigment gene into which a recombinant vector is transformed and the claim must be amended to appropriately convey this concept. This is incorrect. The specification on page 8, lines 18-23 states: "It will be understood that the methods of the present invention are not limited to a particular order for obtaining the *Bacillus* mutant cell. The modification of the gene(s) involved in the production of the red pigment may be introduced into the parent cell at any step in the construction of the cell for the production of a biological substance. It is preferable that the *Bacillus* mutant cell has already been made red pigment-deficient prior to the introduction of a gene(s) directing synthesis of a heterologous biological substance."

Ground 6: The Office Action states that claims 4, 9, 23, 28, 42 and 47 are vague and indefinite because it is unclear if the biopolymer and the metabolite are proteins, but since the method/host cell recited in claim 1/20 is a recombinant protein production method, claims 4, 9, 23, and 29 must recite that the metabolite/biopolymers are proteins.

Claims 4, 9, 23, 28, 42 and 47 have been amended to recite: "the heterologous protein encoded by the first nucleic acid sequence is involved in the biosynthesis of ..."

Ground 7: The Office Action states that the term "heterologous biological substance" in claim 20 is vague and indefinite because it is unclear what substance other than a protein could be made from the claimed transformed host. This rejection is respectfully traversed for the same reasons described under Ground 1 above.

Ground 8: The Office Action states that the phrase "comprising a modification of at least one of the genes *cypX* and *yvmC*" in claim 20 is vague and confusing because the claim should provide structural properties, *e.g.*, *cypX* comprising the nucleotide sequence set forth in SEQ ID NO:1 and *yvmC* comprising the nucleotides sequence of SEQ ID NO:8, to adequately define the genes.

Applicants have amended claim 20 in a similar manner described for claim 1 under Ground 2 above.

Ground 9: The Office Action states that the term "modification" in claim 20 should be changed to "mutation" and specifically recite that the mutation results in loss of red pigment production because claim 1 does not directly correlate the mutation to the loss of pigment.

Claim 20 has been amended according to the Examiner's suggestion.

Ground 10: The Office Action states that claim 20 is also vague and indefinite because it reads on using a cell with unmodified *cypX* or *yvmC* genes transformed with a vector comprising mutant *cypX*/*yvmC* genes and reads as if the desired substance to be produced is actually linked to the mutated *cypX* or *yvmC* gene, when the specification teaches it is a cell comprising a mutated red pigment gene into which a recombinant vector is transformed and the claim must be amended to appropriately convey this concept. This rejection is respectfully traversed for the same reasons described under Ground 5 above.

Ground 11: The Office Action states that the term "heterologous biological substance" in claim 39 is vague and indefinite because it is unclear what substance other than a protein could be made from the claimed transformed host. This rejection is respectfully traversed for the same reasons described under Ground 1 above.

Ground 12: The Office Action states that the phrase "comprising a modification of at least one of the genes *cypX* and *yvmC*" in claim 39 is vague and confusing because the claim should provide structural properties, *e.g.*, *cypX* comprising the nucleotide sequence set forth in SEQ ID NO:1 and *yvmC* comprising the nucleotides sequence of SEQ ID NO:8, to adequately define the genes.

Applicants have amended claim 39 in a similar manner described for claim 1 under Ground 2 above.

Ground 13: The Office Action states that the term "modification" in claim 39 should be changed to "mutation" and specifically recite that the mutation results in loss of red pigment production because claim 1 does not directly correlate the mutation to the loss of pigment.

Claim 39 has been amended according to the Examiner's suggestions.

Ground 14: The Office Action states that claim 39 is also vague and indefinite because it reads on using a cell with unmodified *cypX* or *yvmC* genes transformed with a vector comprising mutant *cypX*/*yvmC* genes and reads as if the desired substance to be produced is actually linked to the mutated *cypX* or *yvmC* gene, when the specification teaches it is a cell comprising a mutated red pigment gene into which a recombinant vector is transformed and the claim must be amended to appropriately convey this concept. This rejection is respectfully

traversed for the same reasons described under Ground 5 above.

For the foregoing reasons, Applicants submit that the claims overcome the rejections under 35 U.S.C. § 112. Applicants respectfully request reconsideration and withdrawal of the rejection.

II. The Rejection of Claims 1-4, 9, 12, 15, 20-23, 28, 31, 34, 39-42, 47, and 50 under 35 U.S.C. § 112, First Paragraph

Claims 1-4, 9, 12, 15, 20-23, 28, 31, 34, 39-42, 47, and 50 stand rejected under 35 U.S.C. § 112, first paragraph, as failing to comply with the enablement requirement. The Office Action stated:

> [T]he specification, while being enabling for "A method of producing a heterologous protein, comprising: transforming a mutant *B. subtilis* cell, wherein said mutant cell comprises a mutation in the *cypX* gene comprising SEQ ID NO:1 or the *yvmC* gene comprising SEQ ID NO: 7, in which said mutation renders the cell deficient in red pigment compared to a wild-type *B. subtilis* cell comprising said *cypX* gene comprising SEQ ID NO:1 or the *yvmC* gene comprising SEQ ID NO: 7, with a recombinant vector comprising a nucleic acid directing synthesis *of* the heterologous protein and recovering the heterologous protein from the cell"; "a mutant *B. subtilis* cell, wherein said mutant cell comprises a mutation in the *cypX* gene comprising SEQ ID NO:1 or the *yvmC* gene comprising SEQ ID NO: 7, in which said mutation renders the cell deficient in red pigment compared to a wild-type cell comprising said *cypX* gene comprising SEQ ID NO:1 or the *yvmC* gene comprising SEQ ID NO: 7, and a recombinant vector comprising a nucleic acid directing synthesis *of* a heterologous protein"; and "A method *of* obtaining a mutant *B. subtilis* cell, comprising: making a mutation to the *cypX* gene comprising SEQ ID NO:1 or the *yvmC* gene comprising SEQ ID NO: 7, in which said mutation renders the cell deficient in red pigment compared to a wild-type *B. subtilis* cell comprising said *cypX* gene comprising SEQ ID NO:1 or the *yvmC* gene comprising SEQ ID NO: 7", does not reasonably provide enablement for the scope of the instant claims.

This rejection is respectfully traversed.

Applicants submit that the specification complies with the enablement requirement.

It is well settled that "[t]he first paragraph of section 112 requires nothing more than objective enablement. How such a teaching is set forth, either by the use of illustrative examples or by broad terminology, is of no importance." *In re Marzocchi*, 169 USPQ 367, 369 (CCPA 1971). Moreover, "a specification disclosure which contains a teaching of the manner and process of making and using the invention in terms which correspond in scope to those used in describing and defining the subject matter sought to be patented must be taken as in compliance with the

enabling requirement of the first paragraph of section 112 unless there is reason to doubt the objective truth of the statements contained therein which must be relied on for enabling support." *In re Marzocchi*, 169 USPQ at 369.

"The determination of what constitutes undue experimentation in a given case requires the application of a standard of reasonableness, having due regard for the nature of the invention and the state of the art ... The test is not quantitative, since a considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed to enable the determination of how to practice a desired embodiment of the invention claimed ..." *Ex parte Jackson*, 217 U.S.P.Q. 804 (Bd. Pat. App. 1982).

It is also well settled that an assertion by the Patent Office that the enabling disclosure is not commensurate in scope with the protection sought must be supported by evidence or reasoning substantiating the doubts so expressed. *In re Dinh-Nguyen*, 181 U.S.P.Q. 46 (C.C.P.A. 1974). See also *U.S. v. Telectronics*, 8 U.S.P.Q.2d 1217 (Fed. Cir. 1988); *In re Bowen*, 181 U.S.P.Q. 48 (C.C.P.A. 1974); *Ex parte Hitzeman*, 9 U.S.P.Q.2d 1821 (BPAI 1988).

Preliminarily, Applicants respectfully point out that the nucleotide sequence of the *Bacillus subtilis yvmC* gene is found in SEQ ID NO: 3. SEQ ID NO: 7 is the nucleotide sequence of the *yvmA* gene.

The reasoning provided in the Office Action is that the specification is enabled only for methods which use *Bacillus subtilis* genes and mutations of the *cypX* gene set forth in SEQ ID NO:1 and the *yvmC* gene set forth in SEQ ID NO:7 and it would require undue experimentation to discover new red pigment genes in any of the other 14 species of *Bacillus*. We respectfully disagree with this assertion. Applicants have shown in Example 6 that primers based on the *cypX* gene from *Bacillus subtilis* were used to clone by PCR the *cypX* gene from *Bacillus licheniformis* and delete a portion of the gene to prevent formation of the red pigment. Consequently, one of ordinary skill in the art can use the *cypX* and *yvmC* genes isolated from *Bacillus subtilis* (see Examples 1 and 2) to synthesize primers based on either the *cypX* gene or *yvmC* gene from *Bacillus subtilis* to clone by PCR the corresponding genes from other *Bacillus species* and delete a portion of such genes to prevent formation of the red pigment.

Furthermore, the specification contains an extensive disclosure of techniques which are well known in the art and indeed routine for persons of ordinary skill in the art for identifying

and using other *cypX* and *yvmC* genes. Applicants describe methods for identifying *cypX-yvmC* operons using DNA microarrays (Example 1); for isolating *cypX-yvmC* operons from *Bacillus subtilis* and *Bacillus licheniformis* (Examples 2 and 6); for constructing *Bacillus subtilis* strains and *Bacillus licheniformis* strains with disrupted *cypX-yvmC* operons (Examples 2-4, Example 6; and page 4, line 24, to page 7, line 10, of the specification); for fermenting *Bacillus* strains to show the absence of red pigment formation; for determining the degree of homology between two nucleic acid sequences using the Wilbur-Lipman method according to Wilbur and Lipman, 1983, *Proceedings of the National Academy of Science USA* 80: 726-730 (page 12, line 29, to page 13, line 8); and for expressing heterologous biological substances in red pigment-deficient *Bacillus* strains (page 11, lines 33, to page 17, line 19). It is well within the skill of the art to discover new red pigment genes in other species of *Bacillus* using Applicants' disclosure.

For the foregoing reasons, Applicants submit that the new claims overcome the rejections under 35 U.S.C. § 112. Applicants respectfully request reconsideration and withdrawal of the rejection.

III. The Rejection of Claims 1-4, 9, 12, 15, 20-23, 28, 31, 34, 39-42, 47, and 50 under 35 U.S.C. § 112, First Paragraph

Claims 1-4, 9, 12, 15, 20-23, 28, 31, 34, 39-42, 47, and 50 stand rejected under 35 U.S.C. § 112, first paragraph, as failing to comply with the written description requirement. The Office Action stated:

> The instant specification has only taught that the *cypX* gene set forth in SEQ ID NO: 1 and the *yvmC* gene set forth in SEQ ID NO: 7 are responsible for the production of red pigment in *Bacillus subtilis* cells. The specification also teaches that the red pigment formation is not desirable and must be removed during the recovery and purification of a recombinant protein from the cell or the pigment may co-purify with the protein. It is taught that often cells that have the desirable trait of increased protein expression and secretion possess these red pigment genes. The specification only teaches the *cypX* gene set forth in SEQ ID NO: 1 and the *yvmC* gene set forth in SEQ ID NO: 7 from *Bacillus subtilis.* It is unclear and unpredictable whether the other 14 species *of Bacillus* recited in claims 12, 31 and 50 possess red pigment genes, much less red pigment genes with the sequences set forth in SEQ ID NOs: 1 and 7. The specification only provides adequate written description for methods which use *B. subtilis* genes and mutations of the *cypX* gene set forth in SEQ ID NO: 1 and the *yvmC* gene set forth in SEQ ID NO: 7 and not the broad scope of the claims.

This rejection is respectfully traversed.

Applicants submit that the specification complies with the written description requirement.

It is well settled that "[t]he test for determining compliance with the written description requirement is whether the disclosure of the application as originally filed reasonably conveys to the artisan that the inventor had possession at that time of the later claimed subject matter ..." *In re Kaslow*, 217 USPQ 1089, 1096 (Fed. Cir. 1983).

The Office Action asserts that the specification only provides adequate written description for methods which use *B. subtilis* genes and mutations of the *cypX* gene set forth in SEQ ID NO: 1 and the *yvmC* gene set forth in SEQ ID NO: 7 and it is unclear and unpredictable whether the other 14 species *of Bacillus* recited in claims 12, 31 and 50 possess red pigment genes, much less red pigment genes with the sequences set forth in SEQ ID NOs: 1 and 7.

We disagree with the above assertion for the same reasons discussed in Section II above.

For the foregoing reasons, Applicants submit that the new claims overcome the rejections under 35 U.S.C. § 112. Applicants respectfully request reconsideration and withdrawal of the rejection.

IV. Conclusion

In view of the above, it is respectfully submitted that all claims are in condition for allowance. Early action to that end is respectfully requested. The Examiner is hereby invited to contact the undersigned by telephone if there are any questions concerning this amendment or application.

Respectfully submitted,

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